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Disc Gel Electrophoresis of Blood Sera and Muscle Extracts from some Catostomid Fishes

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Disc Gel Electrophoresis of Blood Sera and Muscle Extracts from some Catostomid Fishes[1]

GENE R. HUNTSMAN

Disc electrophoresis was used to examine blood sera and low ionic strength muscle extracts from specimens of *Ictiobus cyprinellus, I. bubalus, I. niger, Carpiodes carpio, C. velifer, C. cyprinus, Erimyzon oblongus, Minytrema melanops, Moxostoma erythrurum, M. macrolepidotum, Hypentelium nigricans,* and *Catostomus commersonii.* Serum patterns were too variable for use in taxonomy. Muscle extract patterns were quantitatively distinct for each species except the two *Moxostoma.* Variation in technique and in size of fish possibly had slight effects on the patterns. Sex showed no observable effect. Fish of the same species from different areas were shown in some instances to have quantitatively different patterns indicating that electrophoresis of muscle extracts might be useful in differentiating racial stocks. The relation of these patterns was in general agreement with accepted notions of catostomid taxonomy. Patterns indicated that *Ictiobus cyprinellus* and *I. niger* are more closely related to each other than either is to *I. bubalus* and that, of the species examined, *I. bubalus* and *Carpiodes carpio* are most closely related to the ictiobine stock.

INTRODUCTION

CATOSTOMIDS are a suitable group for studies in biochemical taxonomy. Since much of their taxonomy has been well worked out on morphological, anatomical, and meristic characters, a good background exists by which to evaluate the findings of biochemical studies. There are, however, many interesting taxonomic problems in the Catostomidae to which the application of biochemical studies may be of real value.

Carpsuckers, of the genus *Carpiodes,* are among the most numerous fishes in many Iowa streams. Their morphology is highly plastic, and identification of some adult individuals and of small specimens is difficult, if not impossible, by ordinary means (Hubbs *et al.,* 1943; Trautman, 1957). The identification problems encountered in this genus have inspired this study in biochemical taxonomy.

Electrophoresis, a technique that separates proteins on the basis of their ionic charges, permits the study of protein mixtures for taxonomic purposes. Fish hemoglobins, eye-lens proteins, serum proteins, and muscle extracts have shown some taxonomic value. Of these, serum proteins and muscle extracts seem most useful and are studied in this work. Tsuyuki *et al.* (1967), using starch gel electrophoresis, examined the muscle myogen patterns of eight catostomid fishes: *Catostomus catostomus, C. commersonii, Moxostoma anisurum, M. erythrurum, M. macrolepidotum, Minytrema melanops, Carpiodes cyprinus,* and *Ictiobus bubalus.*

MATERIALS AND METHODS

Fishes for electrophoretic studies were collected with electrofishing gear and with seines. Numbers in parentheses following the species names indicate the collection station numbers: *Ictiobus cyprinellus* (Valenciennes), bigmouth buffalo, (1, 2, 4, 12, 18, 19). *Ictiobus niger* (Rafinesque), black buffalo, (8, 20, 21). *Ictiobus bubalus* (Rafinesque), smallmouth buffalo, (1, 8, 12, 19). *Carpiodes carpio* (Rafinesque), river carpsucker, (1, 2, 5, 6, 7, 9, 13, 15). *Carpiodes velifer* (Rafinesque), highfin carpsucker, (1, 4, 5, 6, 7, 9, 18). *Carpiodes cyprinus* (Lesueur), quillback, (1, 3, 4, 5, 6, 7, 9, 13,

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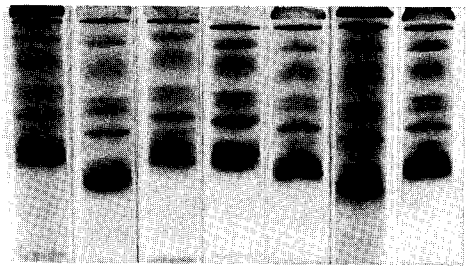

Fig. 1. Disc gels of RCS A serum (a control) from batches 21, 26, 27, 29, 32, 33, and 37. (The bands do not always match horizontally because of differences in running times.)

17). *Minytrema melanops* (Rafinesque), spotted sucker, (15, 22, 23). *Erimyzon oblongus* (Mitchill), creek chubsucker, (16). *Moxostoma erythrurum* (Rafinesque), golden redhorse, (1, 2, 4, 5, 9, 10, 13, 14). *Moxostoma macrolepidotum* (Lesueur), northern redhorse, (3, 4, 6, 9, 12, 14). *Hypentelium nigricans* (Lesueur), northern hogsucker, (9, 10, 11, 13). *Catostomus commersonii* (Lacépède), white sucker, (4, 6, 12, 13).

Dr. Fred P. Meyer, U. S. Bureau of Sports Fisheries and Wildlife, Fish Farming Experimental Station, Stuttgart, Arkansas, identified the buffalo fishes from Arkansas and Dr. Jerome V. Shireman, University of Southwestern Louisiana, Lafayette, Louisiana, identified the spotted suckers from Louisiana. Other determinations were made by field partners or myself.

Collections were made at the following sites and dates: 1–8) Des Moines River, Boone Co., Iowa, on 24 November 1964; 19 August 1965; 27 August 1965; 10 September 1965; 2 October 1965; 23 October 1965; 27 October 1965; 29 December 1965; respectively. 9) Maquoketa River, Delaware Co., Iowa, 6 October 1965. 10) Little Piney River, Phelps Co., Missouri, 30 October 1965. 11) Castor River, Bollinger Co., Missouri, 30 October 1965. 12) Big Sioux and Missouri Rivers, Woodbury Co., Iowa, 3 November 1965. 13) South Fork of the Iowa River, Hardin Co., Iowa, 9 November 1965. 14) Iowa River, Franklin Co., Iowa, 12 November 1965. 15) Mississippi River, Clayton Co., Iowa, 23 November 1965. 16) Big Kinkaid Creek, Jackson Co., Illinois, 26 November 1965. 17) Bluff Creek, Boone Co., Iowa, October 1965. 18) Des Moines River, Boone Co., Iowa, 1 August 1966. 19) Mississippi River, Lee Co., Iowa, 2 August 1966. 20) Ponds. Fish Farming Experimental Station, Bureau of Sport Fisheries and Wildlife, Stuttgart, Arkansas Co., Arkansas, 9 August 1966. 21) Arkansas River, Arkansas, 9 August 1966. 22) Comite River, East Feliciana Parish, Louisiana, 12 July 1967. 23) Indian Creek, Rapides Parish, Louisiana, 15 July 1967.

Sex and total length were recorded for the 187 fish sampled for blood, or muscle, or usually both. Blood was taken by cardiac puncture, allowed to clot, and centrifuged, and the resulting serum was kept frozen until used.

Muscle samples were collected and frozen in the field and kept frozen until used. Muscle samples were homogenized with twice their volume of 0.05 ionic strength phosphate buffer at pH 7.5 (Connell, 1953). Glassware and buffer were prechilled to 0° C, and the muscle samples remained at least partly frozen until homogenized. After centrifugation the muscle extract was dialyzed against the phosphate buffer for 48 hr. A later experiment with bluestriped grunt (*Haemulon sciurus*) muscle indicated that undialyzed muscle extracts made with distilled water gave the same patterns as dialyzed extracts made with phosphate buffer.

Despite a warning that freezing muscle extracts might change their electrophoretic patterns, paired "t" tests comparing the patterns of frozen and unfrozen extracts from the same individuals showed no effect of freezing, even though a large flocculent precipitate appeared upon thawing frozen extracts. Although most extracts were not frozen, I included information from frozen samples.

Advantages of disc electrophoresis were given by Ornstein (1964). One major disadvantage of disc electrophoresis is that one can study only the anodic or the cathodic portion of a sample in a single run. This study concerns the anodic portions of sucker muscle myogens and sera.

Procedures, reagents, and equipment were essentially those described by Davis (1964). This apparatus permitted 12 simultaneous analyses of samples, representing 11 individual fish and the control sample. These 12 samples constituted a "batch." Fig. 1 illustrates gels of RCS A (a sample of *C. carpio* serum used as the control) from batches 21, 26, 27, 29, 32, 33, and 37 and the reproducibility of patterns between batches.

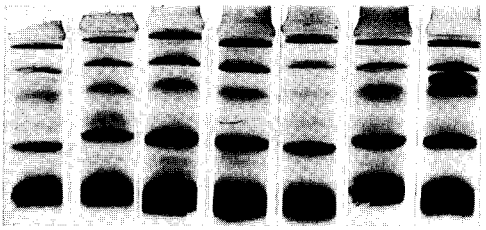

Fig. 2. Six "normal" (left) and one aberrant (right) electrophoretic patterns of *Moxostoma macrolepidotum* serum. Note the single band near the origin in the "normal" pattern that is replaced by two in the other.

Sample doses per gel were 4–5 μliters of serum 6–8 μliters of muscle extract since the latter were more dilute. For each fish, one gel was made for muscle extract and one for serum. A Buchler model 3-1014A regulated power supply was used. A current of 5 ma per tube was passed through the gel tubes until the marker dye, a 0.001% bromphenol blue solution used as an internal standard, was about 5 mm from the end of the tube (55–75 min).

After electrophoresis gels were removed from the gel tubes, stained with amido black, and destained electrophoretically. Destained gels were stored in 7% acetic acid and scanned with a Photovolt Densicord Densitometer (model 5099). A Photovolt Integraph recorded the area under the curves on the resulting strip chart as a series of blips. Percentage protein in each band was calculated by dividing the number of blips under a curve by the total number of blips. Measurements of the gels were used to separate bands on the tracings where such separations were indistinct.

Electrophoresis of Serum Proteins

For most species, the wide range of intraspecific variability of disc electrophoretic patterns of serum proteins prohibited the discernment of a typical species pattern. Patterns of a single species could sometimes be grouped into two or three groups that were internally similar, but such grouping was subjective, and intermediate patterns often existed.

A species-specific pattern appeared to exist for the northern redhorse, *M. macrolepidotum*. The 16 fish examined were of both sexes, ranged from 254 to 427 mm TL, and were from four widely separated collecting sites, the Des Moines, Iowa, Big Sioux, and Maquoketa rivers. Of these 16, only one fish, a 384-mm female from the Big Sioux, showed a pattern difference. Fig. 2 shows six "normal" northern redhorse patterns, on the left, and the aberrant pattern on the right. The single band near the origin in the usual patterns is replaced by two bands in the aberrant pattern. Though a member of the same subgenus as the northern redhorse, the golden redhorse hardly had any two patterns alike. This phenomenon is unexplained.

Serum proteins respond to genetic, physiological, and environmental factors (Thomas and McCrimmon, 1964; Booke, 1964, 1965; Bouck and Ball, 1965; Tsuyuki and Roberts, 1965). Disc electrophoretic patterns of serum proteins seem too sensitive to these effects for use in catostomid taxonomy. Less sensitive techniques, such as paper electrophoresis, seem to provide a better tool for taxonomic studies of serum proteins. Disc gel electrophoresis may be useful in studies within a species because of its high resolution.

Electrophoresis of Muscle Extracts

Disc electrophoretic patterns of sucker muscle extracts revealed up to five main bands and several smaller bands which tended to remain near the origin (Figs. 3–5). Homologies between species were decided on the basis of equal migration of the bands in gels of the same batch in which the marker dye had moved equal distances. Equal migration does not guarantee that two substances are the same, but in these patterns (obtained by identical procedures and from closely related species), I assumed that like migration indicated homology. In all species examined, except *H. nigricans*, all the main bands are homologous to main bands in other species. The hogsucker pattern exhibits, in addition to main bands shared by other species, a prominent band near the origin, designated the Z band, which may or may not be homologous to one of the minor bands in other species.

Because collections of *C. carpio* muscle samples obtained specifically for testing intraspecific effects were lost, I tested for these effects by using collections from other species where these latter collections offered appropriate contrasts. Since these collections were not designed for this test, sample

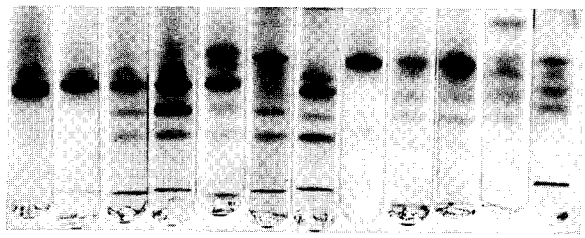

Fig. 3. Disc gel electrophoretic patterns of muscle extracts from *Ictiobus cyprinellus, I. niger, I. bubalus, Carpiodes carpio, C. velifer, C. cyprinus, Erimyzon oblongus, Minytrema melanops, Moxostoma macrolepidotum, M. erythrurum, Hypentelium nigricans, Catostomus commersonii.* Homologous bands do not always match horizontally in this illustration because gels chosen for the illustrations had not all run the same amount of time. Homologies were decided upon by using gels where the migrations of the internal standard were equal.

sizes were often small, effects sometimes confounded, and extraneous uncontrolled variations complicated detection of minor effects. Since this was an exploratory study, I thought that a preliminary evaluation of the sources and magnitudes of intraspecific variation was necessary and that statistical testing would provide useful information despite limitations imposed by the samples.

Because reagents or techniques may have varied from day to day, tests were made to evaluate any batch effect. Means of bands 2, 3, 4, and 5 of seven chubsuckers in batch 32 compared by "t" tests to the respective means for four chubsuckers in batch 34 were not significantly different even at the 0.10 level. Similar tests of the means of bands, 1, 2, 3, and 4 for five white suckers each in batch 28 and batch 31 showed no differences even at the 0.30 level. Again "t" tests of the means of bands Z, 2, 3, and 4, for seven hogsuckers in batch 24, three in batch 25, and three in batch 28 demonstrated no significant differences even at the 0.30 level despite violation of orthogonality rules. However, since a Duncan's multiple range test indicated the hogsucker band 1 means differed at the 0.05 level, I made paired comparison tests on the basis of batches where desirable and possible.

No significant sex differences appeared in tests of five male versus seven female river carpsuckers from the Des Moines River 2 October 1965 or of five male versus six female hogsuckers from the South Fork of the Iowa River, 9 November 1965.

Though length and age are not necessarily well correlated, I believe that length provided a measure of maturity adequate for this study. Scatter diagrams of length versus the percentages of protein in the main bands were made for 14 white suckers, 234–384 mm; 18 quillbacks, 107–437 mm; and 19 river carpsuckers, 165–419 mm. Of these 16 diagrams, only three suggested any length-protein concentration relationship, and only one of these, that for quillback band 4, had a significant regression coefficient, $b = -0.49$. In this particular situation, length effect is confounded with batch and area effects, and the regression cannot be attributed unreservedly to length. Evidence does not indicate any length effect strong enough to influence between-species comparison.

Because fishes from different geographic areas may represent different genetic stocks, or possess differences in their proteins because of environmental factors, statistical tests for area effects were made where possible. Student's "t" tests compared the percentages of protein in the main bands of 13 river carpsuckers from the Des Moines River, 2 October 1965, versus three from the Maquoketa River, 6 October 1965; four white suckers from the Maquoketa, 6 October 1965, versus 10 from the Iowa River, 12 November 1965; four hogsuckers from the Castor River, 30 October 1965, versus 12 from the South Fork of the Iowa, 9 November 1965; six quillbacks from the Des Moines River, 2 October 1965, versus six from the Maquoketa, 6 October 1965; and four northern redhorses from the Big Sioux River, 3 November 1965, versus six from the Iowa River, 12 November 1965.

Of 21 tests, a difference of 3.0% between band 4 means of the South Fork and Castor River hogsuckers was significant at the 0.10 level; a difference of 6.4% between the band $2 + 3$ means (for quantitative comparisons, the region between bands 1 and 4

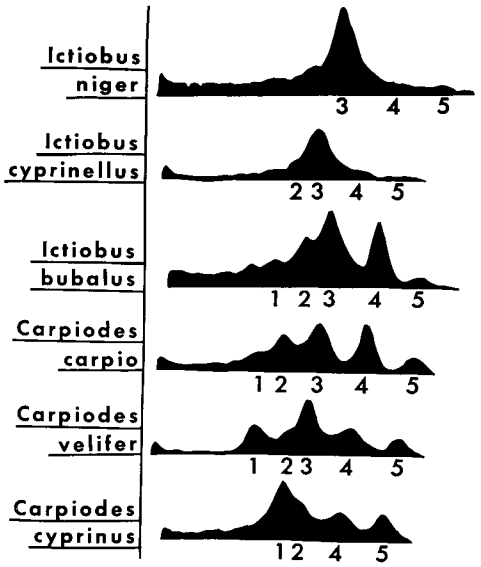

Fig. 4. Representative densitometer tracings of disc elcctrophoretic patterns of muscle extract from some ictiobine fishes.

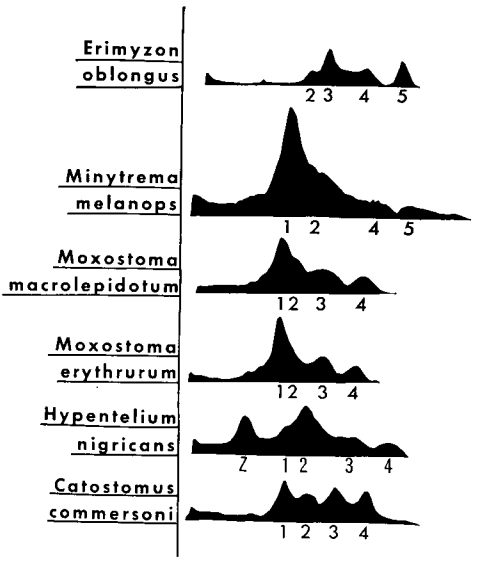

Fig. 5. Representative densitometer tracings of disc electrophoretic patterns from some catostomine fishes.

on the quillback and spotted sucker patterns was designated band 2 + 3 since band 3 is much reduced or missing entirely) of the Des Moines and Maquoketa quillbacks was significant at the 0.05 level; and a difference of 8.5% between Big Sioux and Maquoketa northern redhorse band 4 was significant at the 0.025 level.

Neither Tsuyuki *et al.* (1965) nor I could visually assess geographic differences in electrophoretic patterns of fish muscle extracts. Measurement of the percentage of protein per band seems more sensitive to area differences, and may be of value in the study of racial stocks.

I based most species comparisons on information obtained by combining all the data available for the individual species. Though tests comparing patterns of fish species within collections, or within batches, or within both would be more sensitive than comparisons of the combined samples, I thought that the small sample sizes encountered and the extra effort involved would negate any advantages derived by "within" comparisons. The larger sample sizes achieved by combining the data were statistically desirable. Intraspecific factors that above tests have shown might affect the patterns seem minor and probably can be safely assumed to be allotted randomly.

Information, from the combined data, on the means, maximum and minimum values, standard deviations, and standard errors of the percentages of protein, and the sample size for each band for each species is given in Table 1.

I used Duncan's multiple range test (Steel and Torrie, 1960) with Kramer's (1956) modification for unequal sample sizes (Table 2) for statistical tests of differences between species means. This procedure avoids the sample size demands and the non-orthogonality of Hubbs-Hubbs diagrams (Hubbs and Hubbs, 1953) and is somewhat less conservative than Tukey's test as espoused by Rothschild (1963). These tests of species differences are still inherently conservative because: (1) pooling the data for each species increased the within species variability and decreased the tests' sensitivity; (2) heterogeneity of variance in the tests made them less sensitive; (3) Kramer's modification decreases the sensitivity of Duncan's test if, as sometimes here, sample sizes are greatly unequal. Species differences are shown in spite of this conservatism.

In addition to the tests described, paired comparisons were made of golden and northern redhorse samples in the same batches.

TABLE 1. MEAN, MINIMUM, AND MAXIMUM VALUES, STANDARD DEVIATIONS, STANDARD ERROR, AND ASSOCIATED SAMPLE SIZES FOR THE PERCENTAGES OF PROTEIN FOR THE VARIOUS SUCKERS. (Sample size = number of fish)

Species	$\bar{x}$	No.	Min.	Max.	s	$s_{\bar{x}}$
			Band 1			
Ictiobus niger	4.4	4	0	8.9	5.1	2.5
I. cyprinellus	3.5	11	0	12.6	4.4	1.3
I. bubalus	6.1	6	4.8	9.2	1.6	0.7
Carpiodes carpio	7.9	15	2.9	11.7	2.8	0.7
C. velifer	21.2	7	10.8	30.1	6.5	2.4
C. cyprinus	32.6	21	19.1	71.1	11.4	2.5
Minytrema melanops	46.5	4	43.6	53.3	4.6	2.3
Erimyzon oblongus	1.9	12	0	9.6	3.1	0.9
Moxostoma erythrurum	33.8	14	8.8	56.4	14.1	3.8
M. macrolepidotum	36.0	14	20.2	60.2	11.5	3.1
Hypentelium nigricans	21.3	19	14.9	30.6	4.2	1.0
Catostomus commersonii	23.8	15	18.7	34.8	4.1	1.0
			Band 2			
Ictiobus niger	17.2	3	13.8	19.7	3.1	1.8
I. cyprinellus	11.9	10	5.2	17.1	4.1	1.3
I. bubalus	14.7	7	11.7	18.3	2.1	0.8
Carpiodes carpio	17.6	20	11.9	28.7	3.7	0.8
C. velifer	17.2	6	13.9	20.0	2.4	1.0
*C. cyprinus**	—	—	—	—	—	—
*Minytrema melanops**	—	—	—	—	—	—
Erimyzon oblongus	15.1	11	10.0	23.1	3.8	1.1
Moxostoma erythrurum	18.4	14	8.5	45.9	9.6	2.6
M. macrolepidotum	16.4	14	10.2	24.0	4.1	1.1
Hypentelium nigricans	22.3	19	8.9	31.5	5.9	1.3
Catostomus commersonii	18.3	15	12.2	22.8	2.9	0.7
			Band 3			
Ictiobus niger	44.4	4	34.9	51.5	7.6	3.8
I. cyprinellus	47.9	11	31.4	64.0	9.3	2.8
I. bubalus	39.2	7	29.2	45.9	6.3	2.4
Carpiodes carpio	27.7	20	17.9	37.0	4.5	1.0
C. velifer	34.2	7	29.5	41.9	4.3	1.6
C. cyprinus	—	—	—	—	—	—
Minytrema melanops	—	—	—	—	—	—
Erimyzon oblongus	34.3	11	16.4	42.4	7.4	2.2
Moxostoma erythrurum	19.4	14	8.1	34.3	7.7	2.0
M. macrolepidotum	16.6	14	7.9	24.3	4.4	1.2
Hypentelium nigricans	17.9	19	8.1	23.7	4.2	1.0
Catostomus commersonii	22.7	15	14.8	28.3	3.5	0.9
			Band 4			
Ictiobus niger	7.2	4	1.8	9.6	3.6	1.8
I. cyprinellus	11.0	9	5.9	18.5	4.4	1.5
I. bubalus	15.7	7	12.1	22.2	4.0	1.5
Carpiodes carpio	25.5	20	19.0	30.2	3.1	0.7
C. velifer	11.0	7	5.9	23.8	6.4	2.4
C. cyprinus	10.8	21	1.9	27.0	5.7	1.2
Minytrema melanops	8.2	4	5.8	10.3	1.9	0.9

TABLE 1. *Continued.*

Species	$\bar{x}$	No.	Min.	Max.	s	$s_{\bar{x}}$
			Band 4 (*continued*)			
Erimyzon oblongus	22.3	12	10.0	31.9	7.5	2.2
Moxostoma erythrurum	12.7	14	7.0	27.0	5.2	1.4
M. macrolepidotum	14.5	14	7.2	30.9	6.7	1.8
Hypentelium nigricans	11.6	19	7.5	18.4	2.9	0.7
Catostomus commersonii	20.4	15	9.7	28.4	5.3	1.4
			Band 5			
Ictiobus niger	3.0	4	2.3	4.3	0.9	0.4
I. cyprinellus	5.2	9	2.5	11.9	2.9	1.0
I. bubalus	9.9	7	3.8	12.8	3.3	1.2
Carpiodes carpio	9.7	20	6.0	16.2	3.1	0.7
C. velifer	5.7	7	0.8	9.9	3.5	1.3
C. cyprinus	7.5	21	0.7	14.7	3.4	0.7
Minytrema melanops	6.2	4	4.3	8.0	1.6	0.8
Erimyzon oblongus	15.7	12	8.0	32.4	6.4	1.9
Moxostoma erythrurum	—	—	—	—	—	—
M. macrolepidotum	1.0	14	0.0	6.7	2.2	0.6
Hypentelium nigricans	—	—	—	—	—	—
Catostomus commersonii	—	—	—	—	—	—
			Band 2+3			
Carpiodes carpio	45.4	20	39.5	54.1	4.3	1.0
C. velifer	50.9	6	45.3	60.2	5.3	2.2
C. cyprinus	26.9	21	4.3	38.5	8.0	1.8
Minytrema melanops	14.2	4	9.8	23.8	6.6	3.3

* Band 3 is reduced and difficult to separate from band 2. For comparative purposes the area covered by both is treated as one band.

When more than one sample of one or both species occurred in the same batch, I averaged the values for a species within a batch. These paired comparisons were between averages, averages and single values, or single values. Despite the effort to remove batch effects and despite hiding much variability in the averages, none of the four bands showed a significant difference between the two species.

Some small traces of band 5 were noted in a few northern redhorse extracts but none occurred in golden redhorse extracts. It is possible that a faint trace of band 5 substance may be present in all northern redhorse samples and that this might separate these from those of the golden redhorse. Some of the minor bands might also be important in separating these species.

Table 3 summarizes the differences in patterns between species. Numbers refer to bands that differ.

No species examined had more than one general pattern although variation in intensity of the bands, as herein described, occurred. Polymorphic patterns, such as Tsuyuki *et al.* (1967) obtained by starch-gel electrophoresis of muscle extracts of *C. catostomus,* were not evident but might appear for some species if more individuals were studied.

DISCUSSION

At least one goal of the study has been accomplished. The three species of *Carpiodes* can be adequately distinguished on the basis of electrophoretic patterns of muscle myogens. Their patterns differed enough so that even considerable variation in the patterns would not mask the identity of the sampled fish. Hubbs (1930) indicated that many of the problems encountered in identifying *Carpiodes* specimens were caused by failure of the investigator to evaluate

TABLE 2. DUNCAN'S TEST FOR SPECIES DIFFERENCES[1]

Band 1

Species	CHB	BMB	BB	SMB	RCS	HF	HOG	WHT	QB	GRH	NRH	SPT
No.	12	11	4	6	15	7	19	15	21	14	14	4
$\bar{x}$	1.9	3.5	4.4	6.1	7.9	21.2	21.3	23.8	32.6	33.8	36.0	46.5

Band 2

Species	BMB	SMB	CHB	NRH	HF	BB	RCS	WHT	GRH	HOG
No.	10	7	11	14	6	3	20	15	14	19
$\bar{x}$	11.9	14.7	15.1	16.4	17.2	17.2	17.6	18.3	18.4	22.3

Band 3

Species	NRH	HOG	GRH	WHT	RCS	HF	CHB	SMB	BB	BMB
No.	14	19	14	15	20	7	11	7	4	11
$\bar{x}$	16.6	17.9	19.4	22.7	27.7	34.2	34.3	39.2	44.4	47.9

Band 4

Species	BB	SPT	QB	BMB	HF	HOG	GRH	NRH	SMB	WHT	CHB	RCS
No.	4	4	21	9	7	19	14	14	7	15	12	20
$\bar{x}$	7.2	8.2	10.8	11.0	11.0	11.6	12.7	14.5	15.7	20.4	22.3	25.5

Band 5

Species	NRH	BB	BMB	HF	SPT	QB	RCS	SMB	CHB
No.	14	4	9	7	4	21	20	7	12
$\bar{x}$	1.0	3.0	5.2	5.7	6.2	7.5	9.7	9.9	15.7

Bands 2 + 3

Species	SPT	QB	RCS	HF
No.	4	21	20	6
$\bar{x}$	14.2	26.9	45.4	50.9

[1] Lines drawn under the ranked means indicate groups of means not significantly different from one another. In band 2 the dotted line indicates the situation in which, because of small samples, highfin carpsucker and black buffalo means are not significantly different from the hogsucker mean whereas the river carpsucker, white sucker, and golden redhorse means are different. Initials of the species are those given in Table 3. Differences indicated are significant at the 0.05 level.

the specimen on the basis of several criteria. The muscle myogen pattern should allow identification on the basis of a single technique. Krumholz (1943) thought that the Weberian apparatus of *Carpiodes* species were diagnostic. Nelson (1948) thought, however, that catostomid Weberian apparatus could not be used to distinguish units below the tribal level.

Electrophoretic patterns correlated with

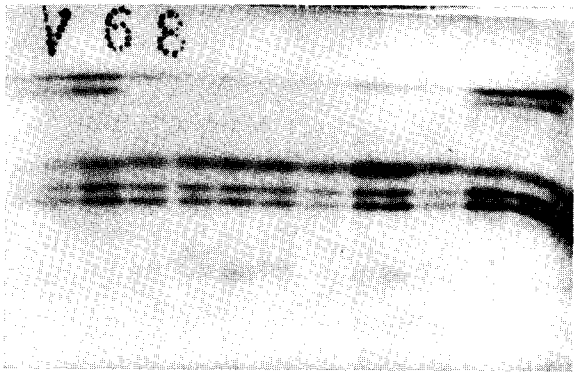

Fig. 6. Starch gel electrophcrogram comparing muscle myogen patterns of three species of *Ictiobus*. From left to right: *I. bubalus, I. bubalus, I. bubalus, I. cyprinellus, I. niger, I. niger, I. niger,* suspected *I. niger* × *I. cyprinellus* hybrid. *I. cyprinellus, I. cyprinellus, I. bubalus, I. bubalus, I. bubalus.*

the accepted relationships in the Catostomidae. On the basis of band 5, the patterns fitted the evolutionary scheme proposed by Miller (1958) and Nelson (1948). All Ictiobinae studied possessed at least a measurable trace of band 5. Both members of the Erimyzonini, which Miller depicted as arising only slightly later than the Ictiobinae from the catostomid stem, also possessed band 5. The Moxostomatini and Catostomini show only a trace (*M. macrolepidotum*) or no evidence (*M. erythrurum, H. nigricans,* and *C. commersonii*) of band 5.

In the Ictiobinae, the two most similar patterns are those of *C. carpio* and of *I. bubalus.* This could indicate that these species, of those studied, are nearest the ictiobine stem stock. The smallmouth buffalo is the most *Carpiodes*-like of the U. S. buffalo suckers and has been named as a *Carpiodes* species at least once (Jordan, 1878). The two Central American buffalofishes have also been called *Carpiodes* species (Meek, 1904). Knowledge of their electrophoretic patterns would be especially interesting.

I. cyprinellus and *I. niger* had disc gel patterns similar to each other's and markedly different from that of *I. bubalus.* To investigate further this unexpected phenomenon, I used starch-gel electrophoresis to compare the muscle extracts of the three buffalo species (Fig. 6). Again *I. cyprinellus* and *I. niger* had nearly identical patterns, while *I. bubalus* had a much different pattern. This evidence does not support the actions of Fowler (1913) and Hubbs (1930) who placed the bigmouth buffalo in a separate subgenus and genus, respectively. Hubbs (1955) later decided the differences merited only subgeneric recognition. Drawings of the Weberian apparatus by Krumholz (1943) show a greater resemblance of *I. bubalus* and *I. niger* apparati to one another than to that of *I. cyprinellus* and support the grouping of *I. bubalus* and *I. niger.* Jordan and Evermann (1896) could not always distinguish *I. niger* (then *urus*) from *I. cyprinellus* and they were not sure that these species were distinct. Though modern authors agree to the validity of *I. niger,* the electrophoretic patterns indicate a close relationship of this species to *I. cyprinellus.* Morphological changes associated with the plankton eating habits (Johnson, 1963) of *I. cyprinellus* may mask a true affinity to *I. niger.*

In *Carpiodes,* the patterns, as well as the morphology, of *C. velifer* and *C. carpio* are most similar. *C. cyprinus* has seemingly secondarily lost band 3 and may be a more "advanced" member of the genus.

In the Erimyzonini, the *M. melanops* pattern, because of the small amount of band 5 present and the predominance of bands 1 and 2, might indicate that this species was closer than *Erimyzon* to the catostomine stock. This would agree with Nelson's (1948) conclusion. If, as Miller (1958) depicted, the Erimyzonini, of the catostomine tribes, arose from the stem stock closest to that point from which the Ictiobinae branched, then the strong band 5 of *Erimyzon* would indicate that this genus is actually less "specialized." Nelson (1948) found the

TABLE 3. BANDS SHOWING A DIFFERENCE BETWEEN SPECIES

	WHT	HOG	NRH	GRH	CHB	SPT	QB	HF	RCS	SMB	BMB
Ictiobus niger	BB[1]	1,3,4,5	1,3,5	1,3,4	1,3,5	3,4,5	1	1,5	1,3	3,4,5	4,5	2
I. cyprinellus	BMB	1,2,3,4,5	1,2,3,5	1,3,5	1,2,3,5	3,4,5	1	1	1,3	2,3,4,5	3,5	
I. bubalus	SMB	1,3,4,5	1,2,3,5	1,3,5	1,3,5	4,5	1,4	1	1	3,4		
Carpiodes carpio	RCS	1,3,4,5	1,2,3,4,5	1,3,4,5	1,3,4,5	3,5	1,2+3,4	1,2+3,4	1,3,4			
C. velifer	HF	3,4,5	3,5	1,3,5	1,3,5	1,4,5	1,2+3	1,2+3				
C. cyprinus	QB	1,4,5	1,5	5	5	1,4,5	1,2+3					
Minytrema melanops	SPT	1,4,5	1,5	1,5	1,5	1,4,5						
Erimyzon oblongus	CHB	1,3,5	1,2,3,4,5	1,3,4,5	1,3,4,5							
Moxostoma erythrurum	GRH	1,4	1,2									
M. macrolepidotum	NRH	1,3,4,5	1,2,5									
Hypentelium nigricans	HOG	2,3,4										
Catostomus commersonii	WHT											

[1] Initial abbreviation.

Weberian apparatus in *Erimyzon* similar to that of the ictiobine fishes but regarded the similarity a result of convergence. Similarity of the electrophoretic patterns of *Erimyzon* muscle extracts to the patterns of ictiobine fishes seems parallel to the similarity of the Weberian apparati. Coincidence of this evidence leads me to believe that *Erimyzon* may actually be the least specialized of the Erimyzonini.

M. macrolepidotum and *M. erythrurum* have the most similar muscle myogen patterns of any of the fishes studied. Since both are members of the same subgenus, *Moxostoma*, the close agreement of patterns is not surprising and strengthens the contention that myogen patterns do reflect phylogenetic relationships.

Qualitative patterns of the main bands of *H. nigricans* and *C. commersonii* are similar to those of *Moxostoma*, but numerous quantitative differences exist between these patterns. Too few moxostomatine and catostomine species were studied to establish tribal trends.

Protein percentages for *Hypentelium* were between those of *Catostomus* and *Moxostoma* and closest to *Moxostoma* for bands 3 and 4. For bands 1 and 2, *Hypentelium* percentages were closest to *Catostomus*, but *Catostomus* was closer to *Moxostoma* than was *Hypentelium*. Available evidence from protein patterns does not resolve whether *Hypentelium* is most closely related to the Moxostomatini or the Catostomini.

The strong Z band of *Hypentelium* was not seen for any other sucker studied. This phenomenon and the overall similarity of the main bands of *Hypentelium, Moxostoma,* and *Catostomus* closely parallel the results of chromatographic studies of sucker body mucus (Huntsman, 1964).

Disc electrophoretic patterns of muscle myogens seem of obvious taxonomic value, for in most cases, they can differentiate species and indicate relationships among those species. In this study, these patterns pointed out potential problems concerning relationships within *Ictiobus* and concerning the relationship of *Hypentelium* to other catostomid genera. Intraspecific pattern variations were much less than those found in serum protein studies but will possibly provide information for racial studies. An intensive quantitative study of these variations within a single species is needed.

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